Supporting information:

Monitoring of Total Type II Pyrethroid Pesticides in Citrus Oils and Water by Converting to A Common Product 3-Phenoxybenzoic Acid

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LC-MS/MS settings

Separation was carried out on an Acquity UPLC® BEH C18 column (2.1x100 mm, 1.7 μm) from Waters (Milford, MA) with a total run time of 12 min. Mobile phase A was 0.1% acetic acid and 5 mM ammonium acetate in a mixture of 20% water in methanol, and mobile phase B was a mixture of 20% acetonitrile in methanol. The gradient started with 10% B constant for 0.5 min, followed by a linear gradient to 100% B in 3.5 min, held for 3 min, returned to 10% B in 2 min, and equilibrated for 2 min. The flow rate was 0.25 mL/min from min 0 to min 4, increased to 0.5 mL/min at min 4.5, held 2.5 min, and return to 0.25 mL/min at min 6.5. An integrated Valco valve (VICI Valco Instrument Co. Inc, Houston, TX) was used to divert the LC flow to the mass spectrometer at 0.5 min and was switched back to waste at 8 min. The injection volume was 2 μL and the column temperature was kept at 40 °C during the analysis.

The mass spectrometer (MS) was operated in the positive ESI mode for individual pyrethroids and negative ESI mode for 3-phenoxybenzoic acid (3-PBA) and 4-fluoro-3-phenoxybenzoic acid (4-F-3PBA). The compound-independent operating conditions, such as capillary voltage, detector voltage,

nebulizer gas, curtain gas, turbo gas and source temperature, were optimized in order to achieve maximum signal sensitivity. The mass spectrometer acquired data from 0.5 to 8 min.

The MRMs (multiple reaction monitoring mode) and compound dependent MS conditions were optimized for each individual pesticide by using a direct infusion technique. To conduct this manual optimization, pyrethroids and 3-PBA standards of various concentrations were prepared in a mixture of acetonitrile, methanol and water (40:40:20) containing 5 mM ammonium acetate and 0.1% acetic acid. A continuous flow of 10 µL/min of solution was delivered to the MS by a Harvard syringe pump (Harvard Apparatus, Holliston, MA), and corresponding parameters were optimized for each compound. The optimized MRM transitions, declustering potential (DP), collision energy (CE), and collision cell exit energy (CXP) are listed in Table S1. Alternatively, automatic optimization of those parameters can be achieved by using the built-in software.

Table S1: MRM transitions and compound dependent MS parameters for selected pyrethroids, 3-PBA and 4-F-3-PBA.

Compounds	Ionization Mode	MRM1 (m/z)	MRM2 (m/z)	DP (V)	EP (V)	CE (V)		CXP (V)	
						MRM1	MRM2	MRM1	MRM2
Deltamethrin	ESI+	523-281	533-506	56	10	23	13	22	29
Acrinathrin	ESI+	559-208	559-181	88	10	23	45	17	22
β-Cyfluthrin	ESI+	451-191	451-127	56	10	21	42	17	14
lambda-Cyhalothrin	ESI+	467-225	467-141	101	10	36	59	29	17
3-PBA	ESI-	213-93	213-169	-82	-10	-30	-17	-13	-16
4-F-3-PBA	ESI-	231-185	231-93	-82	-10	-30	-42	-20	-13

MRM, multiple reaction monitoring mode; DP, declustering potential; EP, entrance potential; CP, collision potential; CXP, collision cell exit energy.

Standard preparation for LC-MS/MS analysis

Individual stock solutions for pyrethroids were diluted and combined into a single solution in acetonitrile at 10 ppm for each compound. Then the standard solution was serially diluted by mobile phase B into five working standards covering the concentration from 50 ppb to 2 ppm. The working standards contained 10% single fold orange oil.

3-PBA stock solution (1000 ppm) was prepared by dissolving the appropriate amount of 98% pure 3-PBA in acetonitrile. The working standards containing 5% citrus oils were prepared the same way as the individual pyrethroids with concentrations ranging from 1 ppb to 100 ppb. Because of the lack of pure 4-F-3-PBA standard at that time, the concentration of 4-F-3-PBA was determined against the 3-PBA standards assuming that their ionization behaviors in the ESI source were similar.

Sample *preparation for individual pyrethroid analysis by LC-MS/MS*

The citrus oil samples were prepared by a simple dilute and filtration process. Citrus oil (100 µL) was diluted with 900 µL of mobile phase B in a 1.8 mL glass vial and vortexed at 3000 rpm for 10 s. Clean solutions were ready for injection while cloudy solutions were filtered through a 0.2 µm nylon syringe filter (Whatman, Florham Park, NJ).

GC-MS analysis

Helium was used as a carrier gas (Airgas, Sacramento, CA). Injection was 1 µL with 4 washes of hexane and ethyl acetate before and after injection. Injection was splitless with the injection port at 250 ºC. Flow rate was 1.0 mL/min. The temperature program had an initial temperature of 150 ºC which was held for 1 min. Temperature was ramped at 8 ºC/min to 280 ºC which was held for 5 min. The total run time was 22.25 min. The transfer line was set at 280 ºC. Mass spectral acquisition was in single ion monitoring (SIM) mode with a 4 min solvent delay. Acquisition included *m/z* 197 and 271 for 2-PBA and 3-PBA. C^{13}-3-PBA was quantified using *m/z* 203 and 277. 4-Fluoro-3-PBA was quantified using

m/z 215 and 289. The retention time of 2-PBA was 7.8 min, with the 3-PBA derivatives had a retention time of 8.8 min as shown in figure S1.

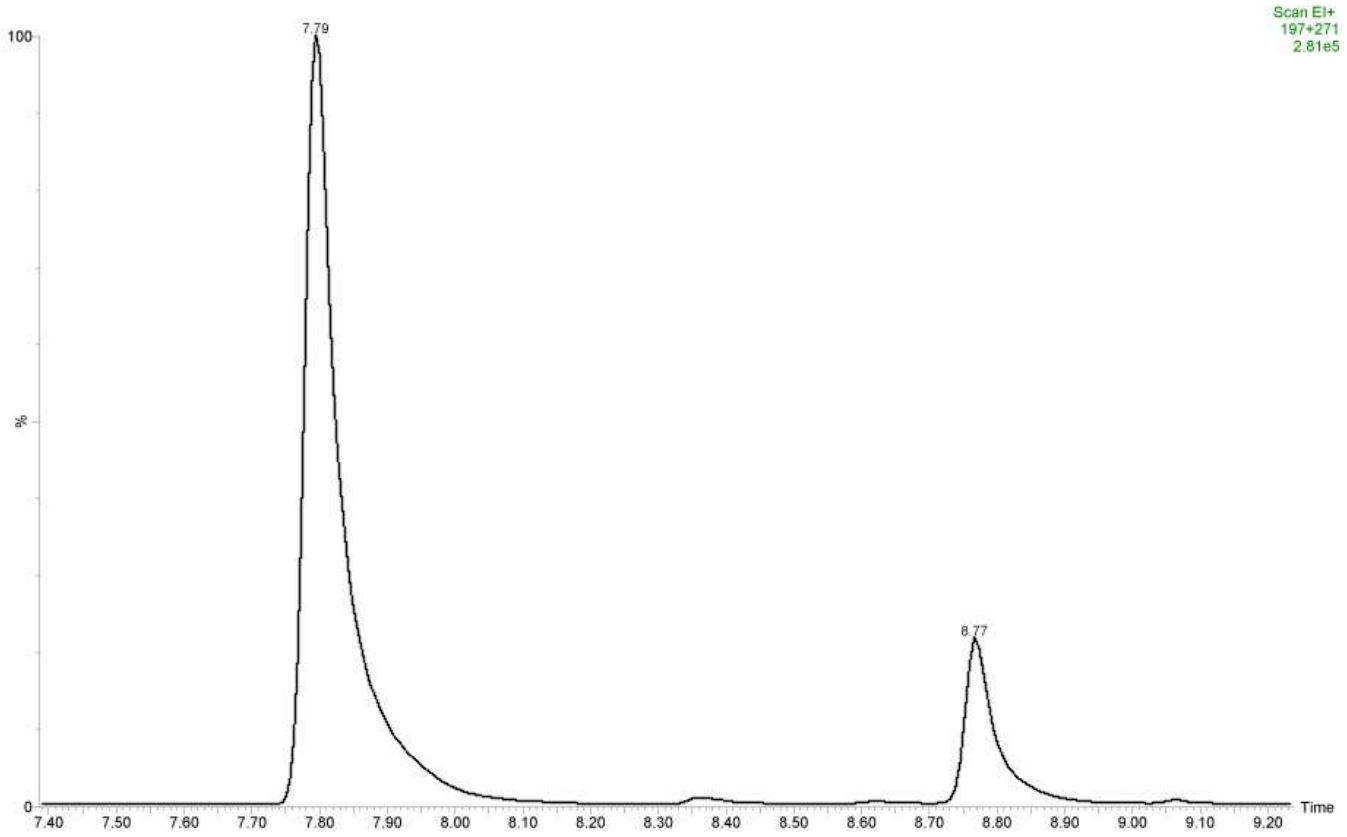

Figure S1: GC-MS chromatogram showing the separation of 2-PBA at 7.8 min and 3-PBA at 8.8 min. Trace is from the selected ion monitoring of m/z 197+271.

Enzyme linked immunosorbent assay (ELISA) for 3-PBA

The ELISA was conducted according to previously reported protocols (Ahn et al., 2011; Shan et al., 2004). The 96 well microplate was prepared by addition of 50 µg/well of 3-PBA conjugated to bovine serum albumin (BSA) in carbonate buffer pH 9.6. The plate was incubated overnight at 4 ºC before it was washed and blocked with 1% BSA (w/v) in phosphate buffered saline (PBS) for 1 h at room temperature. The plate was washed 5x with PBS containing 0.05% Tween 20 (PBST) before use. Standards were prepared by dilution into a certified pesticide free blank sample that had been oxidized and neutralized along with all samples to be analyzed. In the case of Putah Creek water, nanopure water

was used for dilution. A standard curve was prepared by serial dilution starting with a 10 ppm 3-PBA and diluting by a factor of 5. Fifty µL of samples were added in triplicate to the wells of a 96 well plate along with 50 µL of standards in triplicate. Then 50 µL of a 3000 fold dilution of rabbit anti-3-PBA serum in PBST was added. The plate was allowed to incubate for 1 h with shaking before being washed 5x with PBST. A 1:3500 dilution of goat-anti-rabbit-horseradish peroxidase (HRP) conjugate in PBS with 0.5% (w/v) BSA was added to all the wells before the plate was incubated for another hour with shaking. After washing, color was developed by addition of 100 µL of HRP substrate buffer, which was allowed to incubate for 10 min before being stopped by addition of 100 µL of 1 M H_2SO_4. Absorbance readings were taken at 450 nm in a plate reader (Molecular Devices, Sunnyvale, CA).

Solid-phase extraction (SPE) of 3-PBA for instrument analysis

The sample, after neutralization with HCl, was diluted 1:1 with 0.5 M sodium acetate buffer pH 4.5. The Phenomenex Strata-Screen A SPE columns with a 100 mg bed volume were attached to a vaccum manifold and equilibrated with 1 mL of methanol, 1 mL of nanopure water, and 1 mL of acetate buffer prior to use. Columns were loaded with sample followed by washing with 1 mL of water and 1 mL of methanol. The columns were then dried with high vacuum for 10 min. All samples were eluted with 1 mL of 69:30:1 v/v/v hexane:ethyl acetate:glacial acetic acid. The eluate was dried and then spiked with 2-PBA as an internal standard. When analyzed by GC-MS samples, were resuspended in BSTFA derivatization reagent (N,O-bis(trimethylsilyl)trifluoroacetamide).

Test for loss of pyrethroids during volatilization of orange oil under nitrogen at 70 ºC

Cypermethrin with a trace of ^{14}C-cypermethrin was dissolved in methanol. Sixty µL of methanol containing the radioactive cypermethrin was added to 6 vials. The vials were incubated for 15 min to evaporate the methanol before 100 µL of orange oil was added to all vials. Half of the vials were capped, the other half were incubated at 70 ºC for 30 min under a steady stream of nitrogen. After they were cooled, liquid scintillation cocktail was added to all vials and they were counted using a liquid

scintillation counter. There was 5.9 ± 1.6% less radioactivity in the volatilized samples compared to the non-volatilized controls.

Optimization of oxidation conditions

Many oxidants were tried with most being either too strong, which oxidized all of the other components in orange oil without oxidizing the 3-phenoxybenzyl aldehyde, or they were not strong enough to oxidize the 3-phenoxybenzyl aldehyde to completion even in an aquous solution. Three oxidants that we tested were of sufficient reactivity to convert the 3-phenoxybenzyl aldehyde to 3-PBA in the presence of citrus oil: household bleach, sodium chlorite, and hydrogen peroxide. All three were tested to determine the strength needed to perform the oxidation (Figures S2-4).

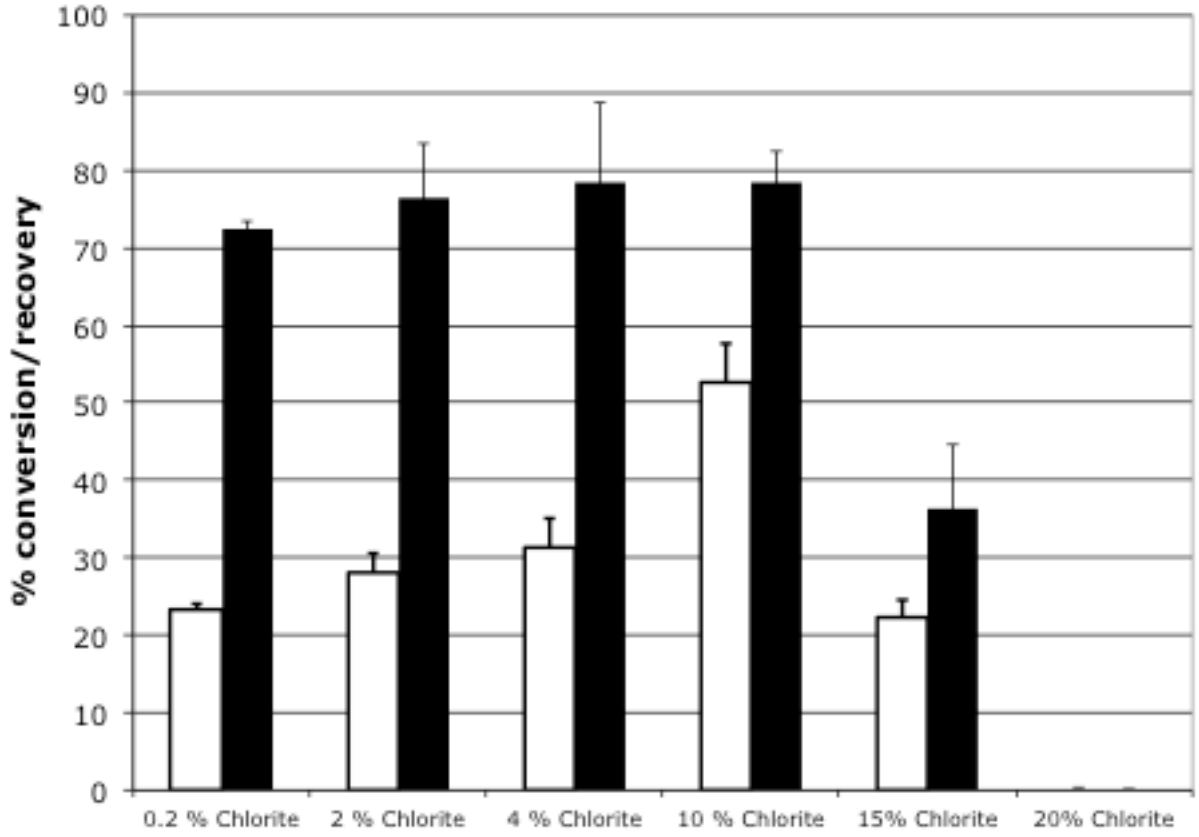

Figure S2: Conversion or recovery of 3-PBA after hydrolysis and oxidation with 0.4 N NaOH and the indicated chlorite concentrations. Samples were spiked with 10 µM deltamethrin in orange oil (white bars). As a recovery standard, samples were also spiked with 10 µM of ^{13}C-3-PBA (black bars). Bars are shown as the average of 4 replicates with error bars being the standard deviation. Samples were analyzed by GC-MS after derivatization with BSTFA. The optimal concentration of chlorite was 10% with a little over 50 % conversion to 3-PBA. Higher concentrations showed chlorination of the 3-PBA causing a loss of the ^{13}C-3-PBA standard.

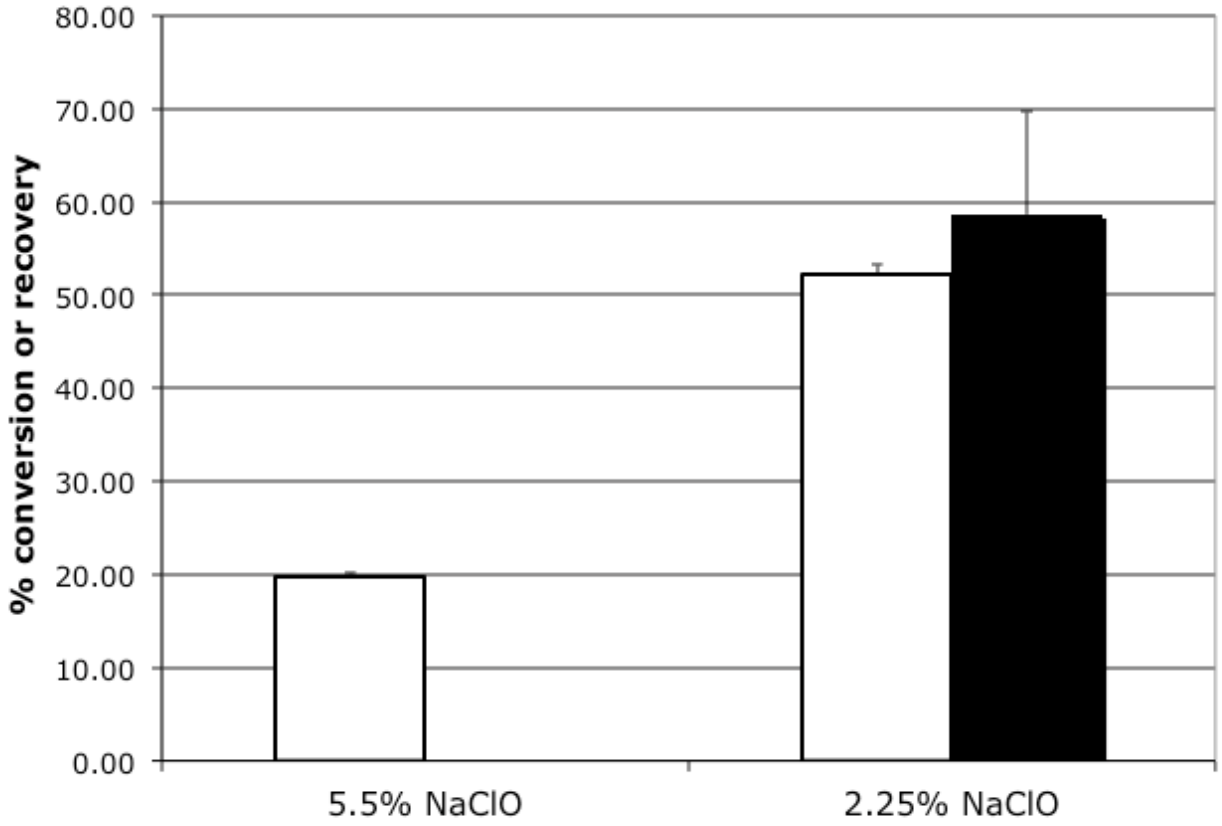

Figure S3: Conversion or recovery of 3-PBA after hydrolysis and oxidation with 0.4 N NaOH and the indicated hypochlorite concentrations (5.5% NaClO was 100% household bleach, 2.25% NaClO was 50% household bleach). Samples were spiked with 10 µM deltamethrin in orange oil (white bars). As a recovery standard, samples were also spiked with 10 µM of ^{13}C-3-PBA (black bars). Bars are shown as the average of 4 replicates with error bars being the standard deviation. Samples were analyzed by GC-MS after derivatization with BSTFA.

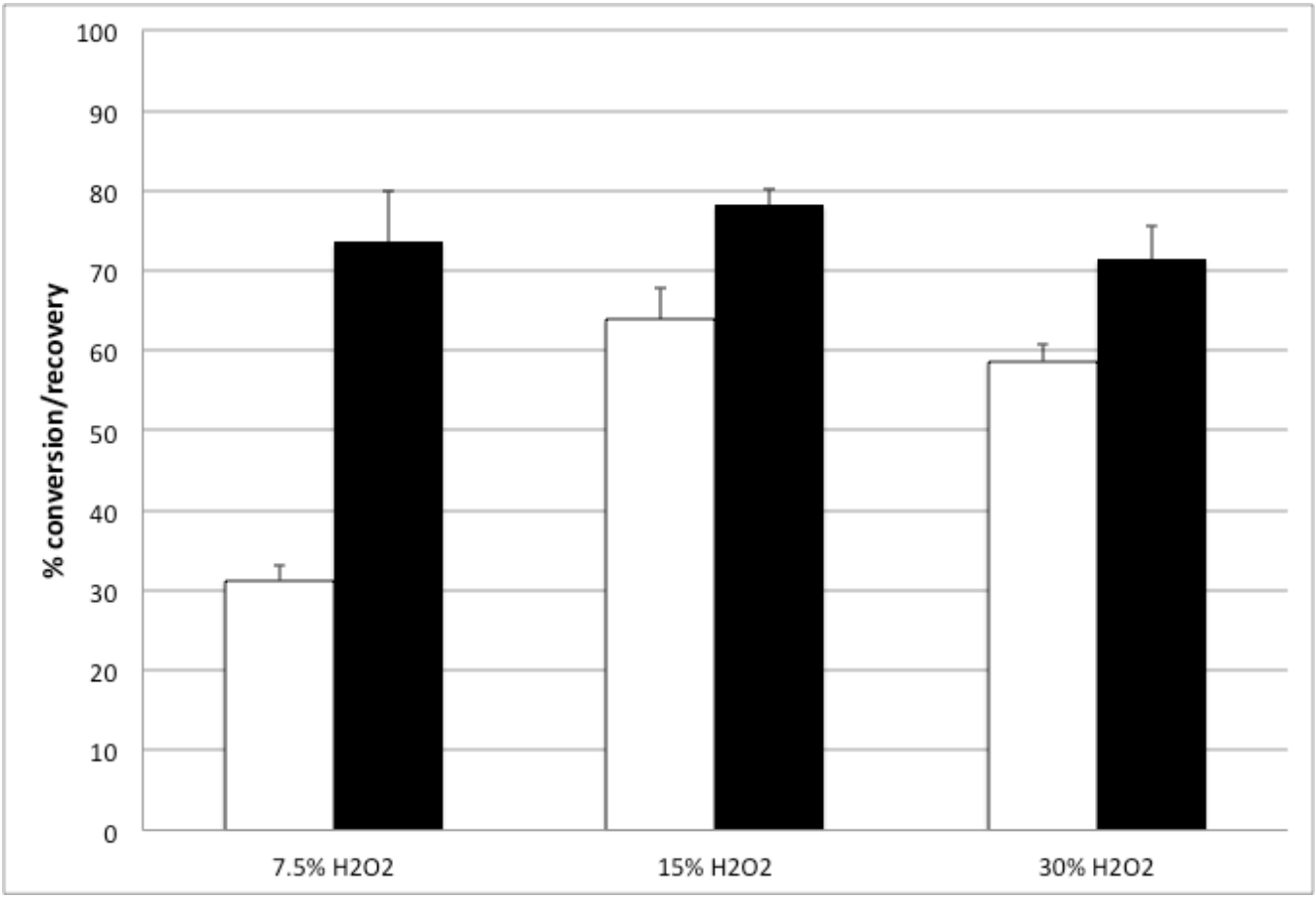

Figure S4: Conversion or recovery of 3-PBA after hydrolysis and oxidation with 0.4 N NaOH and the indicated hydrogen peroxide concentrations. Samples were spiked with 10 µM deltamethrin in orange oil (white bars). As a recovery standard, samples were also spiked with 10 µM of ^{13}C-3-PBA (black bars). Bars are shown as the average of 4 replicates with error bars being the standard deviation. Samples were analyzed by GC-MS after derivatization with BSTFA.

References

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Shan, G., H. Huang, D.W. Stoutamire, S.J. Gee, G. Leng and B.D. Hammock. 2004. A sensitive class-specific immunoassay for the detection of pyrethroid metabolites in human urine. *Chem. Res. Toxicol.* 17:218-225. PMID: 14967009